J. Cotton Res. Dev. **38**(2), 198-206 (July, 2024) **ISSN No. 0972-8619**

Effect of Drought Stress on Biochemical Constituents and Seed Cotton Yield in Upland Cotton

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Abstract : A field experiment was conducted over two consecutive years (2021-2022 and 2022-2023) under both drought and controlled (rainfed) conditions. Seventeen genotypes were evaluated for Relative Water Content (RWC), Specific Leaf Weight (SLW), soluble protein, and proline content, using standard protocols. The results indicated that RWC, SLW seed cotton yield, and average boll weight decreased under water deficit conditions, while soluble protein and proline content increased. These findings suggest that the levels of soluble protein and proline rise with prolonged exposure to stress conditions.

Key words: Drought, proline, reactive oxygen species, RWC, seed cotton yield.

Drought, a significant environmental stressor affecting cultivated lands globally, is known to result in a substantial decline of upto 50 per cent in agricultural yields (Sahitya *et al.*, 2019). The adverse impacts of drought stress on plant growth, development, and overall productivity have been well documented in various studies (Ge *et al.*, 2012; Talaat *et al.*, 2015; Hasan, Alharby, *et al.*, 2020). Crop plants exhibit diverse responses to drought stress, encompassing morphological, physiological, and biochemical adaptations (Hussain *et al.*, 2020; Alabdallah *et al.*, 2021). Reactive Oxygen Species (ROS) production increases under drought stress, posing a threat to plant health. However, plants have evolved antioxidant defense mechanisms and heightened synthesis of antioxidant enzymes to counteract the detrimental effects of ROS (Hasan, Ali, *et al.*, 2020; Hasan *et al.*, 2021; Sohag *et al.*, 2020; Alharbi *et al.*, 2021). Drought stress induces the accumulation of biochemicals such as proline, protein, sucrose, and glycine betaine, contributing to enhanced crop production by mitigating the impact of ROS induced oxidative stress (Perveen and Hussain, 2021). Physiological systems in plants, including cellular respiration, photosynthetic rate, mineral nutrition, enzymatic activity, and Redox homeostasis, are influenced by drought stress regimes. Conditions of water scarcity lead to the degradation of vital biochemicals such as membrane lipo proteins, DNA, and cellular protein composition (Khan *et al.*, 2021).

MATERIALS AND METHODS

The study was conducted on 17 upland cotton genotypes at the cotton research area, CCS Haryana Agricultural University, Hisar. Sowing was done on May 10th, 2021 and May 4th, 2022 by manually dibbling method on the well prepared plots with row to row spacing of 67.5 cm and plant to plant spacing of 30 cm. in a split plot design along with two replications. After sowing no irrigation was given, crop was grown under only rainfed conditions for drought plot, whereas three flood irrigations were given in the control plots.

All the recommended practices were followed to raise a healthy crop. Five plants were tagged in each plot and replications for recording of the observation. The physiological and biochemical constituents were recorded between 60-90 days after sowing and after attaining the less soil water potential as recorded from the drought plot. Relative water content (RWC) and specific leaf weight SLW) were estimated by the method with formula mentioned below.

Initially, the fresh weight of the leaves was measured. The leaves were then placed in 20 ml of distilled water in petri dishes and left for 24 hours in diffused light. After this period, the surface water was carefully blotted off using filter papers, and the turgid weight of the leaves was recorded. Finally, the leaves were dried in an oven and weighed. It was calculated with the following formula:

$$\text{RWC (\%)} = \frac{\text{Fresh Weight - Dry Weight}}{\text{Fully Turgid Weight - Dry Weight}} \quad 100$$

Specific leaf weight was calculated with the following formula:

$$\begin{array}{l}\text{Specific Leaf} \\ \text{Weight (mg cm2)}\end{array} = \frac{\text{Total Dry Weight (g)}}{\text{Total Leaf Area (cm)}} \quad 1000$$

The total soluble protein was estimated using the method developed by Lowry *et al.* (1951), while the proline content was measured using the acid ninhydrin method.

Data of yield attributing characters was recorded from each plot's five tagged plants, and each plot's seed cotton yield was recorded and converted into kilogram per hectare.

RESULTS AND DISCUSSION

The relative water content (RWC) decreased under rainfed conditions compared to irrigated conditions (Fig 1). In 2022, genotypes H 1529, H 1530, H 1581, H 1553, and H 1564 showed a minimal reduction in RWC by 70.49, 69.23, 67.88, 68.58 and 67.36 per cent under rainfed conditions, respectively. In 2023, genotypes H 1530, H 1529, H 1539, H 1480, and H 1564 exhibited a minimal reduction in RWC by 72.53, 76.88, 53.53, 73.72, and 67.40 per cent respectively under rainfed conditions.

The specific leaf weight (SLW) increased in most genotypes as depicted in Fig 2. In 2022, genotypes H 1529, H 1581, H 1564, and H 1530 showed the maximum increase in SLW by 7.13, 6.60, 6.03, and 6.29 (mg/cm2) under rainfed conditions compared to irrigated control. In 2023, genotypes H 1521, H 1528, H 1557, and H 1491 exhibited the highest increase in SLW by 7.15, 6.69, 6.56, and 6.36 (mg/cm2) under rainfed conditions as compared to irrigated control.

As shown in Fig 3, the soluble protein content increased in most genotypes under rainfed conditions compared to irrigated conditions. In 2022, data showed a significant rise in soluble protein across all genotypes under rainfed conditions, with a smaller increase of 3.0, 3.31, and 2.92 per cent observed in genotypes H 1539, H 1530, and H 1480, respectively. In 2023, soluble protein levels generally increased under rainfed conditions for most genotypes but decreased by 1.07, 1.05, 1.17, 1.28, 1.11, and 1.28 per cent in genotypes H 1528, H 1557, H 1569, H 1566, H 1533, and H 1553, respectively.

The proline content increased in most genotypes under rainfed conditions compared to irrigated conditions (Fig 4). In 2022, the highest and lowest proline contents were observed in genotypes H 1521 and H 1539, at 0.86 and 1.61 (µmoles/g) under rainfed conditions respectively. Genotypes H 1529, H 1581, H 1553, H 1530, and H 1569 showed the maximum increases in proline content under rainfed conditions, with values of 1.26, 1.34, 1.28, 1.68, and 1.36 (µmoles/g) respectively as compared to irrigated control. In 2023, proline content increased in most genotypes under rainfed conditions as compared to irrigated conditions. The highest and lowest proline contents were observed in genotypes H 1480 and H 1521, at 1.81 and 0.94 (µmoles/g) under rainfed conditions respectively. Genotypes H 1530, H 1529, H 1581, and H 1564 showed the maximum increase in proline content, with values of 1.76, 1.71, 1.62, and 1.62 (µmoles/g), respectively as compared to irrigated control. The average boll weight was significantly higher in most genotypes when irrigated as shown in Fig 5.

In 2022, the highest seed cotton yields were recorded for genotypes H 1566 under irrigated conditions and H 1530 under rainfed conditions. There is a significant difference in cotton seed yield between irrigated and rainfed conditions. However, the smallest reduction in yield was observed in genotype H 1529, followed by H 1581, H 1553, H 1539, and H 1564. In 2023, genotype H 1530 achieved the highest seed cotton yield under both irrigated and rainfed conditions. The yield under irrigated conditions differed significantly from rainfed conditions, with the smallest decrease seen in H 1530, followed by H 1529, H 1539, H 1547, and H 1553.

Cells play a crucial role in responding to stress by initiating a defense mechanism through changes in gene expression patterns. Stress triggers qualitative and quantitative alterations in proteins, affecting various pathways involved in cellular metabolism and stress defense. Abiotic stresses, such as drought, can lead to protein dysfunction, altering the levels of organization in soluble and structural proteins (Timperio *et al.*, 2008). In a study conducted by Li *et al.* in 2010, the soluble protein content of stem leaves was examined under drought treatments and control conditions. Under control conditions, the soluble protein content gradually declined and remained low

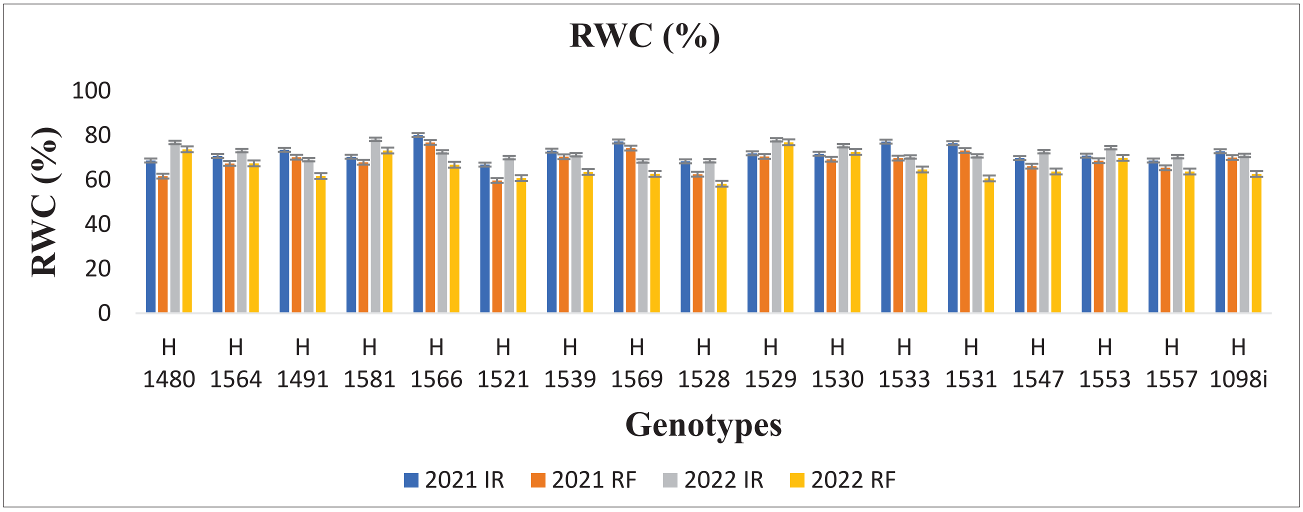

Fig. 1. Effect of drought stress on relative water content in upland cotton plant leaves.

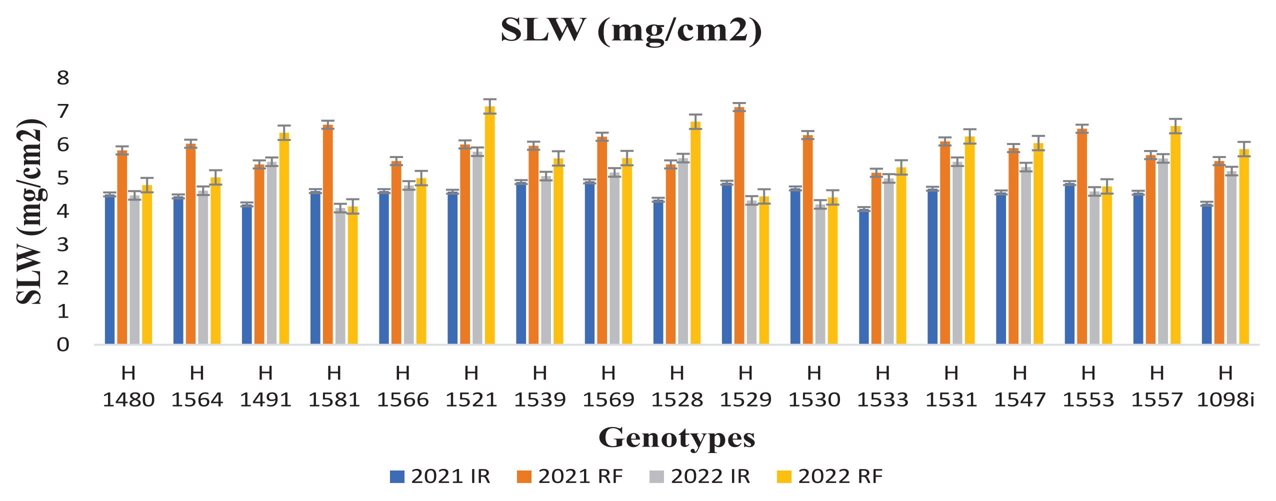

Fig. 2. Effect of drought stress on specific leaf weight in upland cotton plant leaves.

until the final leaf harvest on September 5th. However, in leaves subjected to drought, the soluble protein content initially decreased but significantly rose above that of the control leaves by the final harvest date. Furthermore, on all previous sampling days except during August, the soluble protein levels were notably higher than those of the control, indicating that drought stress might induce an increase in soluble leaf protein content. This elevation could be attributed to reduced water content in plant tissues and heightened levels of osmotic substances under drought stress conditions. Similarly, in our investigation, soluble protein content was observed to increase under rainfed conditions (stress conditions) compared to controlled (irrigated) conditions. This finding correlates with the conclusions of Li *et al.* 2010, suggesting that environmental stressors, like drought, could trigger a rise in soluble protein content as part of the plant's defense mechanism. The increased production of antioxidants, both enzymatic and non-enzymatic, as well as crucial osmoprotectants like proline and various organic

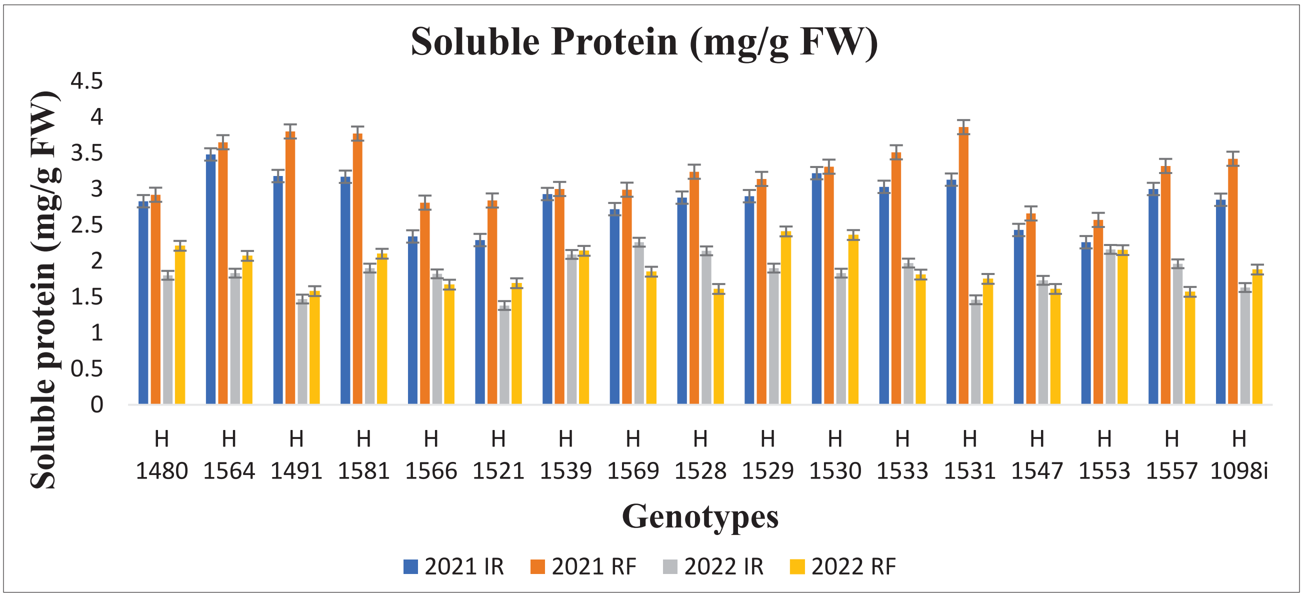

Fig. 3. Effect of drought stress on soluble protein in upland cotton plant leaves.

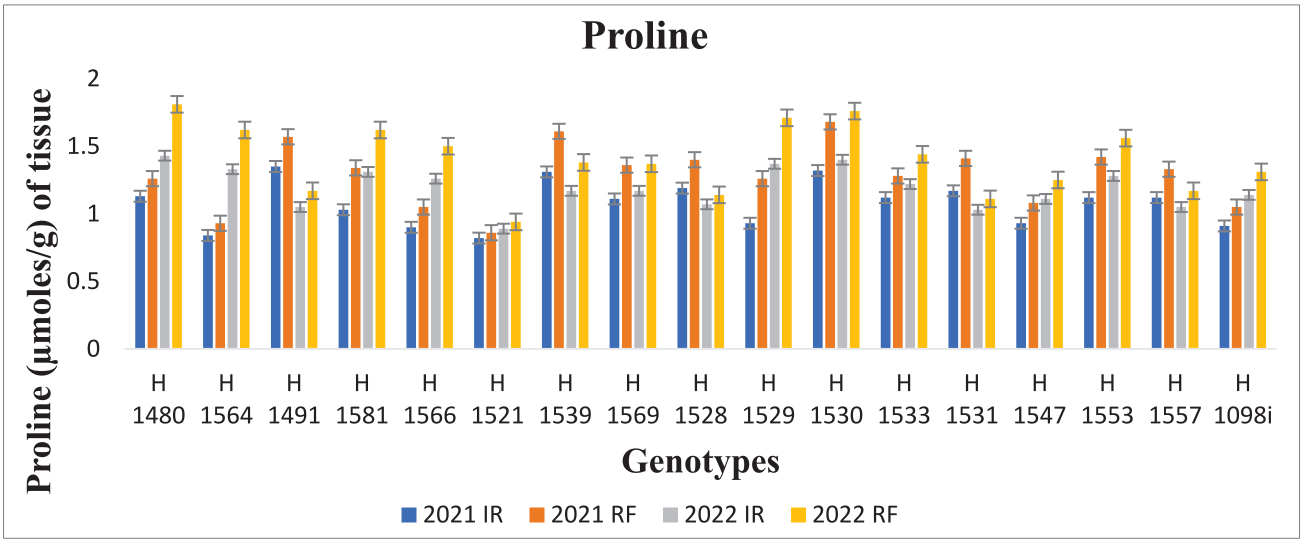

Fig. 4. Effect of drought stress on proline in upland cotton plant leaves.

compounds, represents an adaptive strategy in plants to combat oxidative stress (Zulfiqar *et al.*, 2020; Zulfiqar *et al.*, 2021). Proline, an essential amino acid, is widely present in plants experiencing stressful conditions and plays a crucial role in protein metabolism (Wang *et al.*, 2014; Kavi Kishor *et al.*, 2015; Guan *et al.*, 2020). It contributes to stabilizing proteins and enzymes, storing and transferring metabolic energy, osmoregulation, osmoprotection, metal chelation, and signal transduction (Ashraf & Foolad, 2007; Guan *et al.*, 2020). The levels of proline are affected by the type and severity of stress, as well as differences among species (Delauney and Verma, 1993; Hare and Cress, 1997; Hayat *et al.*, 2012). Acting as a non-enzymatic antioxidant, proline can neutralize singlet oxygen, superoxide, and hydroxyl radicals, contributing to the plant's defense against oxidative stress (Matysik *et al.*, 2002; Szabados and Savouré, 2010; Signorelli, 2016; Rady *et al.*, 2019), although its efficacy in quenching singlet oxygen

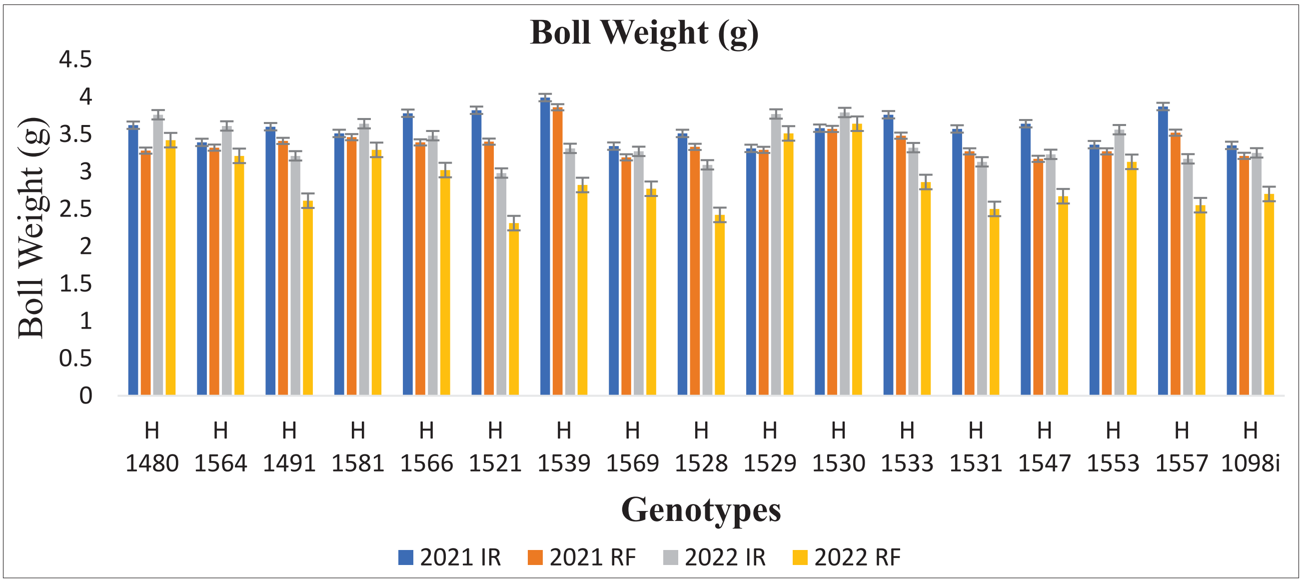

Fig. 5. Effect of drought stress on boll weight in upland cotton.

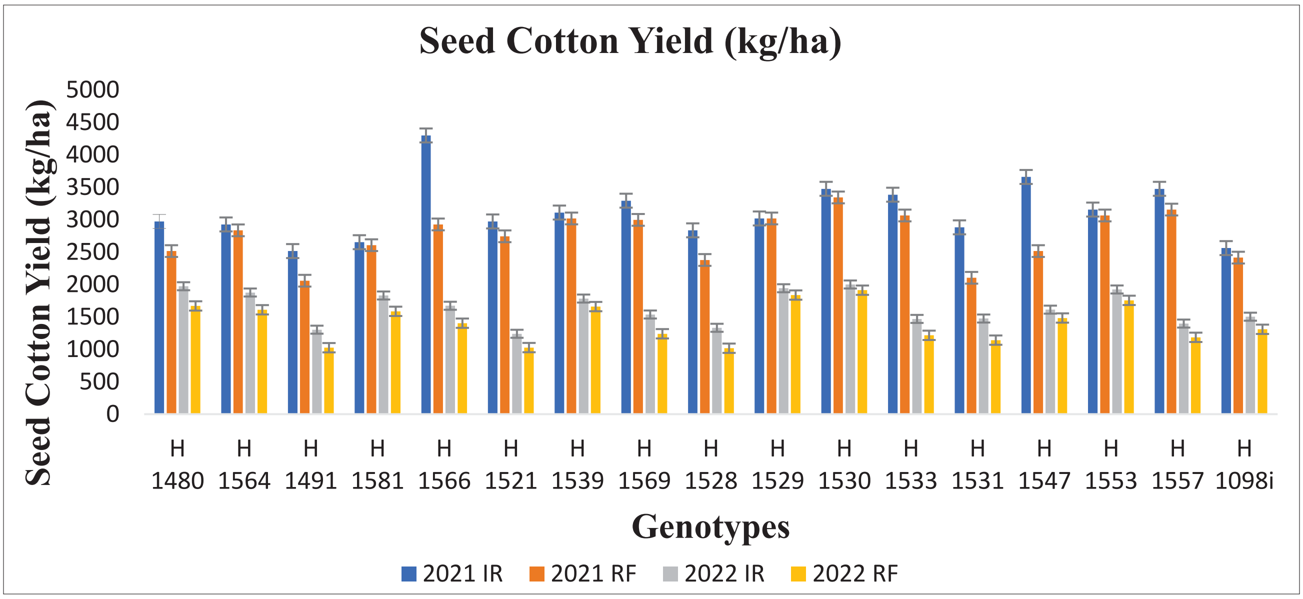

Fig. 6. Effect of drought stress on seed cotton yield in upland cotton.

has been debated. In our current research, we observed an increase in proline content under stressed conditions (rainfed) and lower levels under controlled conditions (irrigated). A similar study by Zameer *et al.*, (2022) noted that proline content was initially low in both transgenic and non-transgenic plants before stress application but increased following stress exposure. Non-transgenic plants exhibited higher proline content at both 0 and 5 days compared to transgenic plants, suggesting a possible correlation between elevated proline levels and reduced membrane stability under stress conditions. Proline, being an osmolyte and organic compound, typically poses no harm to plant cells even at high concentrations. Its synthesis and accumulation patterns vary among plants, with a gradual increase in drought stress triggering proline accumulation in water stressed cotton plants (Zandalinas *et al.*, 2018). In their study on chickpeas, Zandalinas *et al.* (2018) found that transgenic plants exhibited lower proline accumulation than non transgenic plants at 5 days, indicating higher tolerance and survival ability under stress conditions and stability in adverse climates. The relative water content (RWC) is a commonly used indicator for assessing plant water status and is believed to reflect the metabolic rate in tissues. The relationship between RWC and crop yield under water stress conditions further supports this (Anaytullah *et al.*, 2007). The growth of cotton is significantly impacted by a variety of morpho-physiological and metabolic changes induced by drought stress. Severe stress conditions lead to a reduction in plant height, dry matter production, and leaf area index, as well as a decrease in the number of nodes Wang *et al.*, (2017). Dalvi *et al.*, (2019) evaluated 20 cotton genotypes for growth, physiological parameters, and yield parameters under both irrigated and water deficit rainfed conditions, finding a decrease in specific leaf weight under water deficit conditions, which is in accordance with our results. Qian *et al.*, (2020) carried out a lysimeter experiment to examine how different cotton yield indices respond to water stress conditions, which included flooding (for five and eight days), drought (for 10 and 15 days), and a five day flood followed by a 10 day drought during the flowering and boll formation stages. The findings indicated a significant reduction in seed cotton yield across all water stress treatments which is in accordance with our results. Asif *et al.*, (2023) analyzed the morphological, physiological, and fiber quality parameters associated with drought tolerance, employing a comprehensive approach to select superior genotypes from 150 cotton varieties. These were evaluated under both regular and water stressed conditions over two consecutive seasons (2015–2016 and 2016–2017). They found that the average boll weight significantly decreased under water stressed conditions, which is in accordance with our findings. Drought stress poses a significant challenge to crop growth and productivity globally, prompting plants to employ various mechanisms to mitigate its adverse effects. One such adaptive strategy involves the production of antioxidant enzymes and non-antioxidant osmolytic substances like proline and glycine betaine. In our current study, we observed an increase in soluble protein content and proline concentration with escalating drought stress intensity.

CONCLUSION

The growth of cotton is significantly impacted by a variety of morpho physiological and metabolic changes induced by drought stress. Severe stress conditions lead to a reduction in plant height, dry matter production, and leaf area index, as well as a can lead to protein dysfunction, altering the levels of organization in soluble and structural proteins. This elevation could be attributed to reduced water content in plant tissues and heightened levels of osmotic substances under drought-stress conditions. Drought stress poses a significant challenge to crop growth and productivity and plant employ antioxidant like mechanism to mitigate its adverse effect. By employing biotechnological and breeding approaches, we have the potential to enhance the drought tolerance of cotton crops. Thorough and rigorous multi locational trials are essential to evaluate the performance of these genetically modified genotypes.

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Received for publication : January 19, 2024
Accepted for publication : February 26, 2024